REMARKS

Claims 1-7, 10, 12-18, 21, 22, 25, 26, 29-33, 38-44, 47, 51-56, 61-68, 70, 72, 73, 77-80, 82-87, 90-92, 94-96, and 98-103 are currently pending. New claims 104-122 have been added. Claims 1-7, 10, 12, 26, 29-33, 38-44, 47, 51-56, 61-68, 70, 72, 73, 77-80, 82-87, 90-92, 94-96, and 98-100 are cancelled as being directed to non-elected inventions. Applicants expressly reserve the right to file divisional applications directed to these non-elected claims in the future. Claims 13-14, 16-17, 21, 25, and 101-103 are amended herein and new claims 104-122 are added to more particularly claim the subject matter of the present invention.

Sequence Rule Compliance

The sequences found in Figure 1 have been added to the Sequence Listing as SEQ. ID. NOS. 19 and 20. In addition, the paragraph beginning on page 11, line 19 in the Brief Description of the Drawings has been amended to provide sequence identifiers for these sequences. Applicants note that under MPEP §2422.02, where a sequence is given in a Figure, the sequence identifier must be used in the drawing or in the Brief Description of the Drawings.

Objections to the Specification

The specification has been amended to capitalize the trademarks appearing on page 27, line 18; page 33, line 29; page 35, lines 15, 17, and 26; page 36, lines 15 and 16; page 37, line 8; and page 44, line 26 in compliance with MPEP §608.01(v).

Rejections under 35 U.S.C. §112, second paragraph

Reconsideration is requested of the rejection of claims 13-18, 21, 22, 25, and 101-103 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

(a) The Office has objected to the phrase "the combination of a)" in step b of claim 13 as having improper antecedent basis. The phrase "the combination of a)" has been deleted from step b, which now reads "transforming a host cell with the expression vector." Proper antecedent basis for the phrase "the expression vector" can be found in step a ("preparing an expression vector").

(b) Likewise, the Office also objects to the phrase "the combination of a)" in step b of claim 14. The phrase "the combination of a)" has been deleted from step b, which now reads "transforming a host cell with the expression vector." Proper antecedent basis for the phrase "the expression vector" can be found in step a.

(c) The Office has stated that it is unclear what characteristics an expression vector has to have in order to qualify as an "appropriate" expression vector in claims 14 and 25. The term "appropriate" has been deleted from the claims.

(d) The Office has objected to the use of the term "rapidly" in claims 13 and 14 as being indefinite. MPEP §2173.02 indicates that the definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure. The meaning of "rapidly" is clear from the specification--rapid or "sudden dilution of the purified protein solution into a denaturant-free solution allow[s]

the protein to refold without forming insoluble aggregates."[1] The Specification also provides guidelines for determining the meaning of "rapidly."[2]

(e) Step c of claims 13, 14, and 25 has been amended to change "conditions conducive to soluble protein expression" to "conditions conducive to soluble invasin protein expression."

(f) The Office has rejected claims 13, 14, and 25 as vague, indefinite, and confusing in that the claims do not distinctly claim the subject matter which the applicants regard as the invention. In particular, the Office has objected to the phrase "extracting the protein from a host cell lysate, culture medium, or reconstituted organism."

The Office has objected to the phrase "a host cell" in step d. Step d of claims 13, 14, and 25 has been amended to change "a host cell lysate" to "a lysate of the transformed host cell." Proper antecedent basis for "the transformed host cell" may be found in step c of claims 13, 14, and 25.

The Office has also stated that it is unclear how the protein can be extracted from any "culture medium" or "a reconstituted organism," and unclear what is contained in the culture medium and what organism is reconstituted. Step d of claims 13, 14, and

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[1] Specification, p. 28, ln. 19-20. The Specification also states, "Without limiting the invention to any particular theory or mechanism, applicants believe that the rapid removal of denaturant allows beneficial protein *intra-actions*, necessary for correct protein folding, to occur while the rapid dilution of the protein solution minimizes the probability of detrimental protein-protein *interactions*, which form aggregates." *Id.* at ln. 20-24.

[2] "The invasin protein solution, containing the minimum concentration of denaturant, is then rapidly diluted into a buffer containing no denaturant. This process is preferably completed in less than one minute, more preferably in less than 30 seconds, and most preferably in less than 10 seconds." Specification, p. 29, ln. 1-4.

25 has been amended to change "culture medium" to "a culture medium comprising the transformed host cell"[3] and "a reconstituted organism" to "an organism reconstituted from the transformed host cell."[4] The amendments make clear what is contained in the culture medium and what organism is reconstituted. Antecedent basis for "the transformed host cell" can be found in step c of claims 13, 14, and 25.

(g) The phrase "wherein the method of said purification is performed" in step e of claims 13, 14, and 25 has been removed.

(h) Step f of claims 13, 14, and 25 has been amended to change "the denaturant" to "the protein denaturant."

(i) The Office has objected to the phrase "the protein solution" in step f of claims 13, 14, and 25 as having improper antecedent basis. Step f of claims 13, 14, and 25 has been amended to change "the protein solution" to "the purified invasin protein."

(j) The phrase "the purification process of e)" has been removed from step f of claims 13, 14, and 25.

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[3] As noted in the Specification, the host cell may be grown in culture and the invasin protein may be secreted into culture media. *See, e.g.*, Specification, Example 1. *See also* Specification, p. 26-27 (indicating transformed host cells may be grown in culture) and Specification, p. 27, ln. 14.

[4] As noted in the Specification, the protein may be expressed using plant or animal cells which have been reconstituted into whole organisms. Specification, p. 27, ln. 3-4 ("When utilizing plant or animal cells which have been reconstituted into whole organisms...").

(k) Step f of claims 13, 14, and 25 has been amended to change "protein solubility" to "the solubility of the purified invasin protein."

(l) Step g of claims 13, 14, and 25 has been amended to change "the purified protein" to "the purified invasin protein."

(m) Claims 22 and 103 have been amended to change "the purified protein" to "the purified invasin protein."

(n) The Office has stated that "denaturant" as used in the phrase "denaturant-free solution" in step g of claims 13, 14, and 25 is not clear. MPEP §2173.02 indicates that the definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure. The specification clearly defines "denaturant" as "a chemical substance which induces a conformational change in a protein, interfering with protein-protein intra-actions and causing it to lose its tertiary structure. Examples of denaturants are urea and detergents."[5] One of ordinary skill in the art would thus understand what is meant by the phrase "denaturant-free solution," in light of the definition of "denaturant" provided by the specification.

(o) Claim 14, step e has been amended to change "a protein denaturant" to "the protein denaturant." Antecedent basis for the phrase "the protein denaturant" in claim 14, step e and in claims 16 and 17 is found in claim 14, step d.

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[5] Specification, p. 13, ln. 28-30.

(p) Claim 13, step e has been amended to change "a protein denaturant" to "the protein denaturant." Antecedent basis for the phrase "the protein denaturant" in claim 13, step e and in claims 101 and 102 is found in claim 13, step d.

(q) Claim 21 has been amended to add the phrase "after step e, f, or g" to clarify that the affinity purification moiety may be removed from the recombinant invasin protein after any of these steps.[6]

(r) The Office has objected to the use of the term "moiety" in claims 14 and 21 "because it is unclear what is contained in this limitation." As previously discussed, the definiteness of claim language must be analyzed in light of the content of the particular application disclosure.[7] The phrase actually used in claims 14 and 21 is "affinity purification moiety." The specification defines "affinity purification moiety" as "moiety that has been added to a protein in order to allow the protein to be purified using some affinity purification scheme."[8] Furthermore, the specification gives several examples of affinity purification systems that may be used.[9] One skilled in the art

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[6] Support for this amendment is found in the Specification, p. 28, ln. 4-5, which states that "After the protein has been purified, or at a later step, the affinity purification moiety may be selectively cleaved from the recombinant invasin protein..."

[7] MPEP §2173.02.

[8] Specification, p. 13, ln. 22-24. The specification further states, "This portion of the protein may or may not be cleaved from the protein after purification. An example of an affinity purification moiety is the poly-histidine nickel-chelating amino acid sequence described in U.S. Patent No. 5,594,115 and commercially available under the name His-Tag® (Novagen, Madison, WI)." *Id.* at ln. 22-27.

[9] For example, the specification describes affinity purification systems based on fusion proteins which contain metal chelating amino acid sequences, and purification systems using a peptide ligand of a ribonuclease, peptides recognized by specific bound antibodies, a peptide that binds to cellulose, or a peptide which binds to biotin *in vivo*, which then binds as a complex to a streptavidin-coated matrix. See Specification,

would thus understand what is meant by the phrase "affinity purification moiety" in light of the definition and examples provided by the specification.

(s) In light of the foregoing, applicants submit that claims 15-18, 21, 22, and 101-103 are not indefinite because of their dependency on claims 13 or 14.

Rejections under 35 U.S.C. §102(b)

Reconsideration is requested of the rejection of: (i) claims 13-18, 21, 22, and 101-103 under 35 U.S.C. §102(b) as being anticipated by Paul, et al.; (ii) claims 13-16, 21, 22, 101, and 103 under 35 U.S.C. §102(b) as being anticipated by Picking, et al.; and (iii) claims 13, 14, 16, 22, 101, and 103 under 35 U.S.C. §102(b) as being anticipated by Leong, et al.

Claim 13 is directed to a method for the production of a purified recombinant invasin protein. The method comprises: a) preparing an expression vector comprising a polynucleotide encoding an invasin protein; b) transforming a host cell with the expression vector; c) growing the transformed host cell under conditions conductive to soluble invasin protein expression; d) extracting the expressed invasin protein from a lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an organism reconstituted from the transformed host cell with a solution comprising a protein denaturant; e) performing an affinity purification of the extracted invasin protein in the presence of the protein denaturant; f) removing said protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein; and g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

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p. 20-23.

Claim 14 is directed to a method for the production of a purified recombinant invasin protein. The method comprises: a) preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety; b) transforming a host cell with the expression vector; c) growing the transformed host cell under conditions conducive to soluble invasin protein expression; d) extracting the expressed invasin protein from a lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an organism reconstituted from the transformed host cell with a solution comprising a protein denaturant; e) performing an affinity purification of the extracted invasin protein in the presence of the protein denaturant; f) removing said protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein; and g) rapidly diluting the purified invasin protein into a volume of denaturant-free solution.

Claims 15-18, 21-22, 101-103, and new claims 104-119 are either directly or indirectly dependent on claims 13 or 14.

1. Paul, et al. (Human Gene Therapy, 8:1253-62 (July 1, 1997))

Paul, et al. describe the fusion of the GAL4 DNA-binding domain with the invasin cell binding, internalization domain of the *Yersinia pseudotuberculosis inv* gene product, invasin, via a flexible protein linker sequence to produce a protein that can be used to deliver DNA specifically to target cells. Paul, et al. describe growing E. coli containing plasmids encoding GAL4, Inv, or GAL4/Inv, and inducing protein expression. The cells were harvested by centrifugation, and cell pellets were resuspended in lysis buffer that contained 6 M guanidine-HCl, sonicated, and filtered. The cell lysates were passed over a HiTrap Chelating column that had been equilibrated in a buffer containing 8 M urea. Fusion proteins containing a hexahistidine tag were eluted. Prior to dialysis pooled fractions containing protein were diluted. The diluted pools were dialyzed against buffer 1 (which contained 6 M urea). The dialysis buffer was sequentially

diluted two-fold in buffer 1 (without urea) eight times at intervals of at least 2 hours. The samples were dialyzed against buffer 2 (which contained no urea or guanidine-HCl). The solutions were removed from the dialysis chamber and centrifuged. The soluble supernatant was decanted and analyzed by SDS-polyacrylamide gel electrophoresis. Protein concentrations were determined and final purity was found to be greater than 90%. After dialysis, 65-78% of the renatured protein was recovered in the soluble fraction after centrifugation.

Paul, et al. do not describe each and every element of claims 13 and 14.[10] Among other things, claims 13 and 14 both require removing the protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein (step f) and rapidly diluting the purified invasin protein into a volume of denaturant-free solution (step g). Paul, et al. disclose no such steps.

Although Paul, et al. make a general statement regarding empirically determining optimal dilutions to avoid aggregation during refolding, there is no mention of removing denaturant to a minimum concentration necessary to maintain solubility of the purified invasin protein, followed by rapid dilution of the purified invasin protein into a volume of denaturant-free solution. In contrast, Paul, et al. describe refolding the proteins by slowly and sequentially dialyzing away the denaturant.[11]

Furthermore, applicants respectfully disagree with the Office's assertion that "rapid" dilution into a denaturant-free solution is inherent in Paul, et al. In relying on inherency, the Office "must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent feature necessarily

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[10] MPEP §2131 states that a claim is anticipated under 35 U.S.C. §102 only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.

[11] *See* Paul, et al., p. 1255, col. 2.

flows from the teachings of the applied prior art."[12] In addition, the fact that a certain result or characteristic may occur or be present in the prior art is not sufficient for inherency.[13] No such showing has been made by the Office as to rapid dilution of purified invasin protein into a volume of denaturant-free solution. In fact, the only evidence supplied by the Office in support of inherency is a statement that the final recombinant invasin product remained soluble and biologically functional.

The protein renaturation process described by Paul, et al., as previously discussed, involves dialyzing the protein-containing samples against buffer 1 (which contained 6 M urea), and then sequentially diluting the dialysis buffer two-fold in buffer 1 (without urea) eight times at intervals of at least 2 hours. The samples are then dialyzed against buffer 2 (without urea or guanidine-HCl).[14] It is clear that Paul, et al. do not perform a rapid dilution into a denaturant-free solution, but rather perform a sequential dilution over the course of at least 16 hours.[15] Step g of claims 13 and 14 (i.e. rapidly diluting the purified invasin protein into a volume of denaturant-free solution) can thus not be said to be inherent in Paul, et al.[16]

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[12] MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original).

[13] See MPEP §2112, citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993).

[14] Paul, et al., at p. 1255, col. 2.

[15] The sequential dilutions were performed eight times at intervals of at least 2 hours, i.e. a period of at least 16 hours.

[16] Applicants further note that the invasins described in Paul, et al. are from *Yersinia pseudotuberculosis*, and are thus different from the invasin proteins described in the present application. It can not therefore be assumed that the invasins of Paul, et al. would have the same characteristics as the invasins of the present invention. The purification strategy for the invasins of Paul, et al. would thus not necessarily be the same as for the invasin proteins of the present invention.

In light of the foregoing, applicants respectfully request withdrawal of the rejection of claims 13 and 14 under 35 U.S.C. §102(b) as being anticipated by Paul, et al. Claims 15-18, 21, 22, and 101-119 are either directly or indirectly dependent on claim 13 or 14 and are thus patentable for the same reasons as set forth above for these claims, as well as for the additional elements they require.

2. Picking, et al. (Protein Expression and Purification, 8:401-408 (1996))

Picking, et al. describe the cloning, expression, and purification of IpaB and IpaC fusion proteins from *E. coli*. More particularly, Picking, et al. attempted to improve the expression of soluble IpaC and IpaB. Picking, et al. prepared plasmids (pET32b) containing specific IpaB and IpaC gene fragments, an N-terminal leader containing thioredoxin, and six tandem histidine residues, and transformed *E. coli* with these plasmids. The *E. coli* were grown, and target protein synthesis was induced. The *E. coli* were collected by centrifugation, and the bacterial pellets were resuspended in HisBind binding buffer containing 0.1% Triton X-100 (v/v) and lysed by sonication. The soluble fraction was collected following centrifugation, and affinity column chromatography was performed using HisBind resin. The HisTag-Ipa fusion protein was eluted from the column with elution buffer, and dialyzed against a solution containing sodium phosphate, NaCl, and glycerol. Picking, et al. purified recombinant IpaB and IpaC to greater than 90% homogeneity using the nickel-chelation resin.

Picking, et al. do not describe each and every element of claims 13 and 14. Among other things, claims 13 and 14 both require removing the protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the invasin protein (step f), and rapidly diluting the purified invasin protein into a volume of denaturant-free solution (step g). Picking, et al. disclose no such steps.

After affinity column chromatography, Picking, et al. elute the HisTag-Ipa fusion protein from the HisBind resin using elution buffer, and dialyze the eluted protein

against a solution containing sodium phosphate, NaCl, and glycerol. Picking, et al. do not describe step f; i.e., removing protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the invasin protein.

Furthermore, applicants respectfully disagree with the Office's assertion that "rapid" dilution into a denaturant-free solution (i.e. step g) is inherent in Picking, et al. As previously discussed, when relying on inherency, the Office "must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent feature necessarily flows from the teachings of the applied prior art."[17] No such showing has been made by the Office as to step g; the Office has merely asserted that rapid dilution into a denaturant-free solution is inherent in Picking, et al. because the final recombinant invasin protein product remained immunologically functional.

As noted in the specification, the rapid dilution into a denaturant free solution allows the protein to refold without forming insoluble aggregates.[18] Picking, et al. make no reference of this rapid dilution step, but rather, improve solubility by synthesizing fusion proteins with a thioredoxin leader. In fact, it is clear from Picking, et al. that it is the presence of the thioredoxin leader that imparts solubility to and promotes proper

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[17] MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient for inherency. See MPEP § 2112, citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993).

[18] Specification, at p. 28, ln. 18-20.

folding of the invasin protein purified therein.[19] Step g of claims 13 and 14 can thus not be said to be inherent in Picking, et al.

In light of the foregoing, applicants submit that claims 13 and 14 are not anticipated by Picking, et al. Claims 15-16, 21-22, 101, and 103-119 depend either directly or indirectly from claim 13 or 14, and are thus patentable for the same reasons as set forth above for claims 13 and 14, as well as for the additional elements they require.

3. Leong, et al. (EMBO J., 9:1979-89 (1990))

Leong, et al. describe the identification of the region of the invasin protein of *Yersinia pseudotuberculosis* responsible for cell recognition. Leong, et al. further describe the production and purification of invasin deletion derivatives (InvΔ53C and InvΔ254). Cells containing plasmids encoding invasin deletion derivatives were grown, and lysed in a French pressure cell. Unlysed cells were removed by centrifugation, and membranous debris was collected by a second round of centrifugation. The conditions used by Leong, et al. resulted in aggregation of approximately 70% of the overproduced carboxyl-terminal fragment of invasin. The pellet containing the aggregated invasin fragment was washed by resuspension in buffer followed by centrifugation, and the aggregated invasin was dissolved by resuspending the pelleted debris in a solution containing 6 M guanidine-HCl. The guanidine-HCl extract was sequentially dialyzed against a solution containing 0.5 M guanidine-HCl, followed by dialysis against a

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[19] "Use of the pET32b vector allows fusion of the Ipa proteins to a leader protein that is highly soluble in the *E. coli* cytoplasm [ ] and thus can act as a chaperone of sorts in promoting proper folding and enhancing the solubility of recombinant fusion proteins." Picking, et al., at p. 404-405. Picking, et al. further state, "To further improve expression of IpaC and IpaB, the gene for each was subcloned into pET32b for synthesis with an N-terminal thioredoxin leader. Thioredoxin leader proteins have been shown to confer a high degree of solubility to fusion proteins [ ] by acting as a chaperone of sorts in enhancing their proper folding." *Id.* at p. 406.

solution containing no guanidine-HCl. The soluble fraction containing invasin was concentrated by precipitation in 35% ammonium sulfate, and the resuspended pellet was dialyzed against a solution containing Tris-HCl and NaCl. Further purification was achieved by loading the dialysate onto a DEAE-cellulose column. The eluted fraction containing the invasin derivatives was then collected.

Leong, et al. do not describe each and every element of claims 13 and 14. Among other things, Leong, et al. do not disclose steps c, e, f, or g of claims 13 and 14.

For instance, step c of claims 13 and 14 calls for growing the transformed host cell under conditions conducive to soluble invasin protein expression. Leong, et al. do not use such conditions since, as previously discussed, the conditions used by Leong, et al. to overproduce the carboxyl-terminal fragment of invasin resulted in approximately 70% of the invasin in aggregates.[20] The aggregated invasin of Leong, et al. then had to be dissolved using 6 M guanidine-HCl.

In addition, step e of claims 13 and 14 calls for performing an affinity purification of the extracted invasin protein in the presence of a protein denaturant. In contrast, Leong, et al. merely describe loading a dialysate containing the invasin onto a DEAE-cellulose column equilibrated with Tris-HCl and NaCl, and eluting with a 50-250 mM linear NaCl gradient.[21] Thus, Leong, et al. do not use a protein denaturant during affinity purification.

Step f of claims 13 and 14 calls for removing the protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the invasin protein.

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[20] *See* Leong, et al., at p. 1987, col. 1. Conditions conducive to soluble invasin protein expression are discussed on pages 26-27 of the Specification.

[21] Leong, et al., at p. 1987, col. 1.

Leong, et al. describe no such step.[22] Furthermore, such a step would not be necessary in the process described in Leong, et al. since, as discussed above, Leong, et al. do not use a protein denaturant during affinity purification.

Step g of claims 13 and 14 calls for rapidly diluting the purified invasin protein into a volume of denaturant-free solution. Such a step is not disclosed in Leong, et al. Furthermore, applicants respectfully disagree with the Office's assertion that the rapid dilution of purified invasin is inherent from the teachings of Leong, et al. The Office has not provided a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent feature necessarily flows from the teachings of the applied prior art;"[23] but has merely states that the final recombinant invasin protein product of Leong, et al. remained biologically functional. Furthermore, applicants respectfully note that it would be unnecessary to rapidly dilute the purified invasin derivative of Leong, et al. into a denaturant-free solution since, as previously discussed, Leong, et al. do not use a protein denaturant during purification on the DEAE-cellulose column (i.e., the invasin is already in a denaturant free solution). Step g of claims 13 and 14 can thus not be said to be inherent in Leong, et al.[24]

In light of the foregoing, applicants respectfully submit that claims 13 and 14 are not anticipated by Leong, et al. Claims 16, 22, 101, and 103-119 depend either directly

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[22] Leong, et al. merely state that after the invasin is eluted from the DEAE-cellulose column, "[t]he fractions containing the invasin derivative were pooled and stored frozen." *Id.*

[23] MPEP §2112, citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original). The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient for inherency. See MPEP § 2112, citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993).

[24] Applicants further note that the invasins described in Leong, et al. are from *Yersinia pseudotuberculosis*, and are thus different from the invasin proteins described in the present application. As discussed above, it can not therefore be assumed that the purification strategy for these invasins would necessarily be the same as for the invasin proteins of the present invention.

or indirectly from claims 13 or 14 and are thus patentable for the same reasons as set forth above for claims 13 and 14, as well as for the additional elements they require.

Rejections under 35 U.S.C. §103(a)

Reconsideration is requested of the rejection of claim 25 under 35 U.S.C. §103(a) as being unpatentable over Oaks, et al. (Clin. Diagnost. Lab. Immunol., 3:242-245 (1996)) in view of Comb, et al. (U.S. Patent No. 5,834,247), and/or Anilionis, et al. (U.S. Patent No. 5,192,338), Thorne (U.S. Patent No. 5,552,294), and Seed (U.S. Patent No. 5,726,293).

Claim 25 is directed to a method for the production of a purified recombinant invasin protein. The method comprises: a) preparing an expression vector comprising a polynucleotide encoding the invasin protein and a polynucleotide encoding an affinity purification moiety; b) transforming a host cell with the expression vector; c) growing the transformed host cell under conditions conducive to soluble invasin protein expression; d) extracting the expressed invasin protein from a lysate of the transformed host cell, a culture medium comprising the transformed host cell, or an organism reconstituted from the transformed host cell with a solution comprising 6 M urea; e) performing an affinity purification of the extracted invasin protein in the presence of a protein denaturant; f) removing said protein denaturant from the purified invasin protein until the concentration of the protein denaturant is at the minimum concentration necessary to maintain the solubility of the purified invasin protein; and g) diluting the purified invasin protein in about 10 seconds or less into a volume of denaturant-free solution.

Oaks, et al. describe the overexpression of a fusion protein consisting of full-length IpaD fused to a leader peptide containing six histidine residues. Oaks, et al. inserted an *ipaD* fragment into the plasmid expression vector pET-15b, and transformed *E. coli* with the plasmid. Overexpression of the *ipaD* fusion gene was induced. The fusion protein was affinity purified by passing a cytosolic extract

containing the recombinant protein over a Ni^{2+} column followed by elution of the IpaD fusion protein with 1 M imidazole.

For a combination of references to render obvious a claimed invention, the Office must show: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) that the prior art reference (or references when combined) teach or suggest all the claim limitations.[25] The Office has not made such a showing.

Initially, applicants note that Oaks, et al. do not describe the use of a protein denaturant during the production and purification of the IpaD, and therefore do not disclose steps d to g of claim 25. Although Oaks, et al. do describe the use of imidazole as an eluant after affinity purification of the recombinant IpaD, applicants respectfully submit that imidazole is not a protein denaturant, as defined in the Specification. The Specification defines denaturant as "a chemical substance which induces a conformational change in a protein, interfering with protein-protein intra-actions and causing it to lose its tertiary structure. Examples of denaturants are urea and detergents."[26] Imidazole is not such a denaturant.

The Office states that the imidazole used in Oaks, et al. qualifies as a protein denaturant in light of Seed, which teaches imidazole to be a mild denaturant. Seed discloses a method for eluting proteins from affinity matrices, and describes imidazole as a mild denaturant which disrupts protein interactions, thereby facilitating the release of proteins bound to affinity ligands.[27] However, Seed does not describe imidazole as being able to induce a conformational change in a protein, interfere with protein-protein intra-actions, or cause a protein to lose its tertiary structure; Seed merely uses

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[25] MPEP §2142.

[26] Specification, p. 13, ln. 28-30 (emphasis added).

[27] U.S. Patent No. 5,726,293, at col. 2, ln. 46-50.

imidazole as an elution reagent to release a protein from an affinity complex during affinity purification.[28]

One skilled in the art reading Oaks, et al. and Seed, would thus not consider imidazole to be a protein denaturant, as used in claim 25. In fact, in the absence of a denaturant such as guanidine or urea, elution of proteins from a purification matrix with imidazole is considered by those skilled in the art to be elution under non-denaturing conditions.[29] Thus, one skilled in the art would consider the production and purification of the recombinant IpaD in Oaks, et al. to be under non-denaturing conditions. Oaks, et al. can not, therefore, be said to describe steps d to g of claim 25.

Furthermore, there is no suggestion or motivation to combine Oaks, et al. with Seed, Comb, et al., Anilionis, et al., and/or Thorne to arrive at claim 25. Since Oaks, et al. do not use a protein denaturant in the production and purification of the IpaD, it therefore follows that no refolding steps would be required in the method described by Oaks, et al. There is thus no motivation in Oaks, et al. for one skilled in the art to modify the method described therein by using a protein denaturant or by performing steps f and g of claim 25. Furthermore, neither Seed, Comb, et al., Anilionis, et al., or Thorne provide such motivation or suggest that imidazole is a protein denaturant (as defined in the Specification).

Thorne merely discloses a method for detecting a virulence-associated factor (VAF) in a sample. The sample is contacted with a VAF releasing solution under conditions which release VAF, and the released VAF is immunochemically detected.

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[28] *Id.* at col. 3, ln. 11-14.

[29] For example, the Novagen product brochure, in describing purification on a HisBind Resin states: "After unbound proteins are washed away, the target protein is recovered by elution with imidazole...The versatile system allows proteins to be purified under gentle, non-denaturing conditions or in the presence of either 6M guanidine or urea." Novagen product brochure, at p. 2 (emphasis added). A Supplemental Information Disclosure Statement submitting the brochure is filed simultaneously herewith.

The VAF may be a surface antigen for invasin (Shigella dysenteriae or invasive *E. coli*),[30] and the immunological enhancing agent may be urea. The preferred concentration range of immunological enhancing agent is from about 0.1M to about 15M.[31]

Anilionis, et al. describe proteins and peptides related to an outer membrane protein of *Haemophilus influenzae*, and recombinant vectors containing nucleotide sequences encoding PBOMP-1 and PBOMP-2 related peptides, proteins and fusion proteins. Anilionis, et al. also describe the solubilization of a PBOMP-2:PBOMP-1 fusion protein using a solution containing 6M urea, and the removal of the urea after solubilization by dialysis against a buffer without urea.[32]

Comb, et al. disclose modified proteins comprising a controllable intervening protein sequence (CIVPS), which is capable of splicing or cleaving the target protein. The CIVPS may be used in purification of the target protein. Comb, et al. describe the use of 6M urea to solubilize a recombinant protein, and the rapid dilution of the proteins to allow refolding of the diluted proteins.[33] Although Comb, et al. do describe the purification of a recombinant protein using a Ni^{2+} affinity resin in the presence of 6M urea,[34] the protein purified in Comb, et al. is not an invasin protein, as described in the present application.

In light of the foregoing, applicants submit that claim 25 is not unpatentable over Oaks, et al. in view of Comb, et al. and/or Anilionis, et al., Thorne, and Seed. New claims 120-122 are either directly or indirectly dependent on claim 25 and are thus

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[30] U.S. Patent No. 5,552,294, at col. 4, ln. 14-15.

[31] *Id.* at col. 7, ln. 60-65.

[32] U.S. Patent No. 5,196,338, at col. 63, ln. 34-40.

[33] U.S. Patent No. 5,834,247, at col. 46-47.

[34] *See, e.g., id.* at col. 46, ln. 54-59.

patentable for the same reasons as set forth above for claim 25 as well as for the additional elements they require.

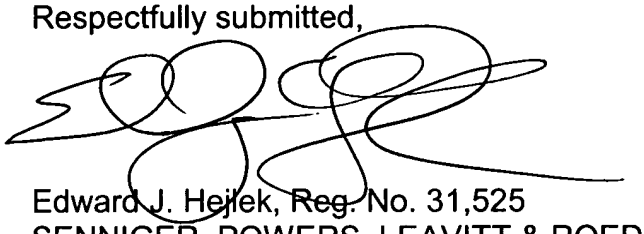
CONCLUSION

In light of the foregoing, applicants respectfully request reconsideration and withdrawal of the rejection of claims 13-18, 21, 22, 25, and 101-103 under 35 U.S.C. 112, second paragraph, the rejection of claims 13-18, 21, 22, and 101-103 under 35 U.S.C. §102(b), and the rejection of claim 25 under 35 U.S.C. §103(a).

Applicants also note that a signed copy of page 1 of applicants' IDS (listing references 1-11) was not included with the signed copy of pages 2-4, and therefore request the Office forward a signed copy of page 1.

Applicants further note that no shortened statutory period for response was indicated in the present office action. Therefore, applicants believe that this reply is timely, having been mailed prior to the end of the six month period for response. Furthermore, applicants believe that no extension fee is due, as no shortened statutory period was indicated. Nonetheless, should applicants' position be incorrect, the Commissioner is hereby authorized to charge any underpayment to Deposit Account No. 19-1345.

Respectfully submitted,



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